Cytochrome P450 Retinoic Acid 4-Hydroxylase Inhibitors: Potential Agents for Cancer Therapy

V.C.O. Njar*

Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, MD 21201-1559, USA.

Abstract: Retinoids play a crucial role in cellular differentiation and proliferation of epithelial tissue and their utility in oncology and dermatology is well documented. This mini review focuses on the role of all-*trans*-retinoic acid (ATRA or RA), the principal endogenous retinoid and its metabolism in cancer therapy.

ATRA has been used successfully in differentiating therapy of acute promyelecytic leukemia and other types of cancers. However, its usefulness is limited by the rapid emergence of ATRA resistance due (in part) to ATRA - induced acceleration of ATRA metabolism. A novel strategy to subjugate the limitation associated with exogenous ATRA therapy has been to modulate and/or increase the levels of endogenous ATRA by inhibiting the cytochrome P450-dependent ATRA-4-hydroxylase enzyme(s) responsible for ATRA metabolism. These inhibitors are also referred to as retinoic acid metabolism blocking agents (RAMBAs). This review highlights development in the design, synthesis and evaluation of RAMBAs since 1987. Major emphasis is given to liarozole, the most studied and only RAMBA to undergo clinical investigation and also the recently developed novel and highly potent 4-azoly retinoids. The potential role of a new family of cytochrome P450 enzymes, CYP26, with specificity towards ATRA is also discussed.

INTRODUCTION

Retinoids (vitamin A and its natural metabolites and synthetic analogs) are currently the subject of intense biological interest stimulated by the discovery and characterization of retinoid receptor and the realization of these compounds as nonsteroidal small-molecule hormones [1]. All-*trans*-retinoic acid (ATRA), the biologically most active metabolite of vitamin A plays a major role in cellular differentiation and proliferation of epithelial tissue. Differentiating agents redirect cells towards their normal phenotype and therefore may reverse or suppress evolving malignant lesions or prevent cancer, and indeed represents an attractive target for medicinal intervention. ATRA is being used in differentiation therapy of cancer, in cancer chemoprevention and for the treatment of acne [2]. Recently, ATRA has proven useful in cancer chemotherapy [3]. One of the most impressive effects of ATRA is on acute promyelocytic leukaemia. Treatment of acute promyelocytic leukaemia patients with high dose of ATRA resulted in complete remission [4, 5]. Furthermore, several experiments in animals have demonstrated that ATRA inhibited the induction and caused the disappearance of prostate tumors [6]. In spite of these encouraging results, the effects of prolonged ATRA therapy on human cancers in the clinic has been scarce and disappointing [7]. It has been suggested that the therapeutic effects of ATRA are undermined by its rapid *in vivo* metabolism and catabolism by cytochrome P450 enzyme(s) [8]. An important consideration is that two cellular retinoic acid binding proteins (referred to as CRABP-I and CRABP-II) are believed to be involved in the presentation of ATRA to metabolizing CYP enzymes [9]. However, a discussion of CRABPs is outside the scope of this review. It should be stated that the two natural isomers of ATRA, 9-cis-retinoic acid (9-CRA) or 13-cis-retinoic acid (13-CRA) are also being investigated for cancer chemoprevention and/or therapy.

One of the strategies for preventing *in vivo* catabolism of ATRA is to inhibit the P450 enzyme(s) responsible for this process. Indeed, this seems to be an emerging approach that may yield effective agents for the chemoprevention and/or treatment of cancers [10]. This review highlights development in the design, synthesis and evaluation of RAMBAs since 1987. Major emphasis is given to liarozole, the most studied and only RAMBA to undergo clinical investigation and also the recently developed novel and potent 4-azoly retinoids. The potential role of a new family of cytochrome P450 enzymes, CYP26, with specificity towards ATRA is also discussed. For recent presentations of work in this field, the review by Miller [10] is recommended. To our knowledge, this represents the first comprehensive review of inhibitors of retinoic acid metabolism enzyme(s).

CYTOCHROME P450 ENZYMES INVOLVED IN ATRA METABOLISM

ATRA is rapidly metabolized by cytochrome P450 (CYP)-dependent enzymes via several routes leading to a

*Address correspondence to this author at the Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, MD 21201-1559, USA. Phone: (410) 706 5885, Fax: (410) 706 0032, E-mail: vnjar001@umaryland.edu

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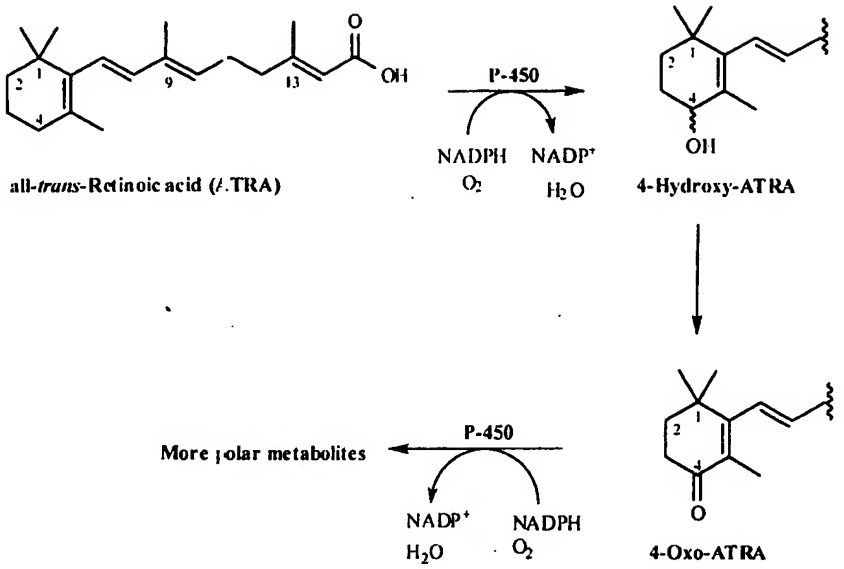


Scheme 1. Metabolic pathway of all-trans retinoic acid.

variety of polar metabolites [11]. However, it is believed that the physiologically most prominent pathway starts with the rate-limiting hydroxylation at C-4 position of the cyclohexenyl ring leading to formation of 4-hydroxy-ATRA. It should be stated that the stereochemistry at C-4 of 4-hydroxy-ATRA is yet to be determined. The latter compound is converted by a reductase enzyme into 4-oxo-ATRA that is then further transformed by CYP(s) into more polar metabolites "Scheme (**1**)" [11]. Although most of these ATRA metabolism studies have been conducted with rodent liver microsomes, similar results have also been obtained using human liver microsomes [12, 13]. Three independent groups have established that of the several human liver CYP isoforms capable of metabolizing ATRA via the 4-hydroxylation route, CYP2C8 is the major contributor, though CYP3A4 and, to a lesser extent CYP2C9, also make contributions [14-16].

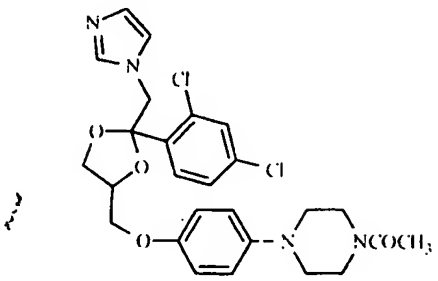
Although several CYPs have been shown to be involved in the catalysis of ATRA 4-hydroxylation, their specificity for ATRA is generally low [14-16]. Recently, a new family of cytochrome P450 enzymes, CYP26A1, has been cloned and characterized in zebra fish, human, and mouse tissues [17-22]. CYP26A1 is ATRA-inducible and appears to be the most dedicated ATRA 4-hydroxylase enzyme known. Interestingly, CYP26A1 does not hydroxylate the closely related 9-CRA or 13-CRA [22, 23]. The enzyme displays high specificity towards ATRA and it may function as an important regulator of differentiation and a possible modulator of disease states by controlling retinoid concentrations and homeostasis. Recent reviews on the cloning and characterization of CYP26A1 have appeared [24, 25]. It should be stated that a new subfamily of the CYP26 family, named CYP26B1, which is 44% identical to CYP26A1 from both mouse and humans has recently been found in humans and zebrafish [26]. Although substrate specificity studies on CYP26B1 are yet to be conducted, it is plausible that this enzyme may recognize 9-CRA and/or 13-CRA, complementing the CYP26A1 activity.

DISTRIBUTION AND ROLE OF CYP26A1

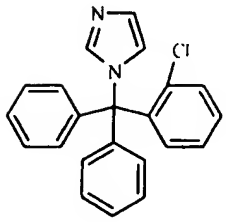
CYP26A1 is expressed in the liver, heart, pituitary gland, adrenal gland, testis and in specific regions of the brain and the placenta [reviewed in 26, 25, 27]. Based on recent studies [25], it is suggested that the major role of CYP26A1 is a protective one, that is, the regulation of intracellular ARTA steady-state levels, exhibiting a similar negative feedback as has been demonstrated for CYP24, which is involved in cholecalciferol catabolism [28]. Although the major retinoid products (4-hydroxy- and 4-oxo-ATRA) of CYP26A1 were originally considered to be inactive retinoids, there is compelling evidence which suggest that they are highly active modulators of positional specification in amphibian embryonic development and they bind and activate retinoic acid receptors (RAR) subtypes as efficiently as ATRA [29, 30]. Thus, in development CYP26A1 may fulfil functions distinct from metabolic inactivation of ATRA.

CYP26A1 is readily induced by ATRA in a variety of normal and cancer cells and the enzyme efficiently converts ATRA into its oxygenated derivatives. Although the therapeutic potential of ATRA has been demonstrated (reviewed in [6, 31]), a major draw back to its clinical application is the prompt emergence of resistance, attributed to the induction of oxidative catabolism through CYPs [8, 32-35], and CYP26A1 could be a major contributor. Because ATRA deficiency is associated with the progression of some cancers [36-38], it is possible that ATRA-induced CYP26A1 is involved in rapid metabolism of ATRA in cancer patients.

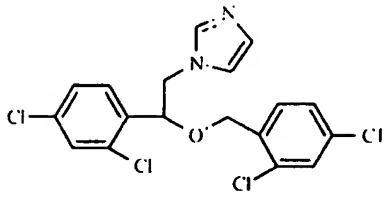
The cloning and characterization of CYP26A1 represents an important development in ATRA (retinoid) biochemistry and molecular biology. The enzyme's inducibility by ATRA, and its ATRA metabolic/catabolic activity defines a feedback loop, which may be critical in regulating both normal and therapeutic levels of ATRA. This emphasizes the importance of maintaining stable physiological levels of



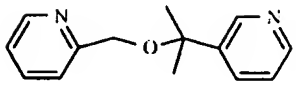
1, ketoconazole



2, Clotrimazole



3, Miconazole



4, Metyrapone

Fig. (1). Structure of antimycotics.

ATRA. Thus, compounds designed to inhibit CYP26A1 activity may be useful in elevating normal tissue ATRA levels or maintaining high therapeutic levels of ATRA. As stated earlier, since ATRA has proven useful in the treatment and/or chemoprevention of some cancers and skin disorders, it is now possible to investigate the contributions of the expression/activity of CYP26 (or lack thereof) in various disease. CYP26 has recently been mapped to human chromosome 10q23-q24 [39], a region where several suppressor gene loci have been described [40] as well as the split-hand-split foot syndrome (SHSF-3) [41]. Thus, it is possible that mutations in CYP26 may play a role in these diseases.

ATRA 4-HYDROXYLASE INHIBITION

The realization that the metabolism of ATRA may be responsible for its limited efficacy in the clinics provided the impetus behind the search for inhibitors of the CYP-mediated metabolism of ATRA. Although the pioneering paper by Napoli's group in 1987 which led the way in this field was greeted with enthusiasm [42], research in the area have been rather slow with only a few inhibitors described over the past 14 years.

Inhibitors of ATRA 4-hydroxylase (also referred to as retinoic acid metabolism blocking agents [RAMBAs]) should delay *in vivo* ATRA catabolism resulting in increased endogenous levels. This effect should improve control of neoplastic differentiation and growth and possibly exhibit antitumor activity.

EARLY STUDIES OF ATRA 4-HYDROXYLASE INHIBITORS

The antimycotics, ketoconazole (keto), clotrimazole, miconazole and metyrapone "Fig. (1)" appear to be the first compounds evaluated as potential inhibitors of ATRA metabolism enzyme(s) [42]. As shown in "Table (1)" keto and clotrimazole were about equipotent, exhibiting potent inhibition of ATRA metabolism in F9 embryonal carcinoma cells. Miconazole had no more than 10% of the activity of clotrimazole, while metyrapone had about 1% of the activity of clotrimazole. The inhibition of ATRA metabolism by most of these compounds is not surprising since they are well known inhibitors of CYP-P450-mediated metabolism.

Following this initial study, Van Wauwe *et al.* [43], in 1988, examined the effects of keto on ATRA metabolism, *in vitro* using hamster liver microsomes and *in vivo* using normal rats treated with [^{3}H]-ATRA. *In vitro*, keto inhibited the CYP-P450-mediated metabolism of ATRA to the corresponding polar 4-hydroxy- and 4-oxo- derivatives. *In vivo*, keto suppressed the formation of polar ATRA metabolites by normal rats dosed intrajugularly with 200 mg of [^{3}H]-ATRA. These studies are significant as they demonstrated for the first time the feasibility to design CYP inhibitors that could prolong the half-life of exogenously administered ATRA to animals. In 1990, this group examined the effects of keto, R75251 (now known as liarozole or liazal ^{TM}) and five other CYP-P450 inhibitors on the *in vivo* metabolism of ATRA in normal rats [44] Keto and R75251 were the only compounds found able to delay the metabolism of exogenously administered ATRA. In addition, liarozole enhanced the endogenous plasma levels of ATRA, exerting ATRA-mimetic effects *in vivo* [45]. It should be noted that the same group of Janssen researchers first reported that CYP-P450-dependent aromatase, 17-hydroxylase/17,20-lyase (CYP17), and 11-hydroxylase were the target enzymes for R75251 [46]. But in another related paper, they also reported that the antitumoral effects of R75251 on the growth of transplantable R3327 prostate adenocarcinoma in rats was independent of its inhibition of androgen biosynthesis [47]. Subsequent studies in this area were mostly conducted by researchers of Janssen Pharmaceutical, who eventually settled on liarozole for development as an anti-cancer agent and for the treatment of

Table 1. Inhibition of Retinoic Acid Metabolism in F9 Embryonal Carcinoma Cells by Antimycotics [42]

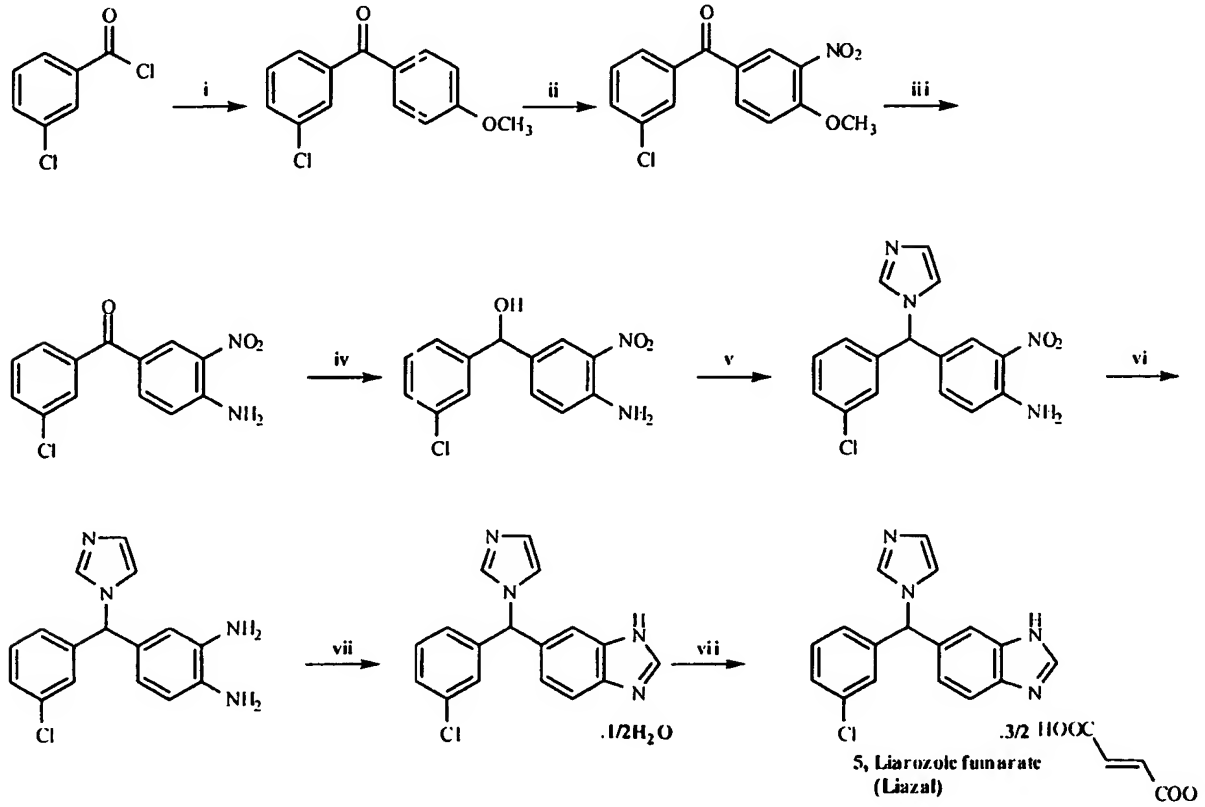
Inhibitor	% Inhibition Concentration (μM)		
	1	10	100
Ketoconazole	42	84	100
Clotrimazole	22	62	84
Miconazole	8	12	56
Metyrapone	0	2	26

dermatological diseases. Liarozole is the most studied inhibitors of ATRA 4-hydroxylase and remains to date the only RAMBA to have been evaluated clinically and, as such, is a standard against which future RAMBAs may be judged. The summary of studies on liarozole and the recent RAMBAs are discussed below.

LIAROZOLE (LIAZAL™, 5-[(3-CHLOROPHYNYL-1H-IMIDAZOLE-1-YL)METHYL]-1H-BENIMIDAZOLE)

Following discovery of the undesirable side-effects of keto (*vide supra*) in PCa patients, the quest for more potent non-steroidal CYP17 inhibitors ensued. Liarozole (Liazal™, 5-[(3-chlorophynyl-1H-imidazole-1-yl)methyl]-1H-benimidazole) is the result of extensive SAR studies on imidazole derivatives [48]. Very recently, a paper has appeared which describes a practical 8-step synthesis of Liazal™ "Scheme (2)" [49].

Liarozole is a good inhibitor of rat CYP17 (IC_{50} = 260 nM) with similar potency to keto (IC_{50} = 340 nM), but unlike keto, it was also a potent inhibitor of aromatase [46]. However, following extensive *in vitro* and *in vivo* studies, liarozole's biological effects are ascribed to inhibition of the P450-dependent 4-hydroxylase that catalyzes ATRA metabolism [50]. *In vitro*, liarozole (IC_{50}, 2.2 μM) suppressed the P-450-mediated conversion of ATRA to more polar metabolites by hamster liver microsomes. *In vivo*, it enhanced the plasma level of ATRA from mostly undectectable values (less than 0.5 ng/ml) in control rats to 1.4 ± 0.1 and 2.9 ± 0.1 ng/ml in animals treated p.o. with 5 and 20 mg/kg of liarozole [45]. Anti-tumoral action was detected in androgen-dependent and androgen-independent rat prostate carcinoma models [47, 51, 52]. Remarkable anti-tumor activity was observed against prostate cancer xenographs in immunodepressed mice [53, 54] and further studies revealed that the anti-tumor properties of liarozole correlates with an increase in tumor differentiation, following accumulation of ATRA [55]. These studies established that the anti-tumoral properties of the compound



Reagents and conditions: i, $PhOCH_3$, $AlCl_3$, CH_2Cl_2, 5-10°C; ii, HNO_3/H_2SO_4, CH_2Cl_2, 10-15°C, 1h; iii, NH_3,g,i. PrOH, 100°C, 16h; iv, $NaBH_4$,i. PrOH, reflux, 1h; v) CDI, CH_2Cl_2, reflux, 1h; vi, H_2, Pt/C 5% thiophene sol., MeOH, rt.; HCOOH, 4N HCl, reflux; viii, EtOH, 50°C, fumaric acid.

Scheme 2. Synthesis of Liarozole.

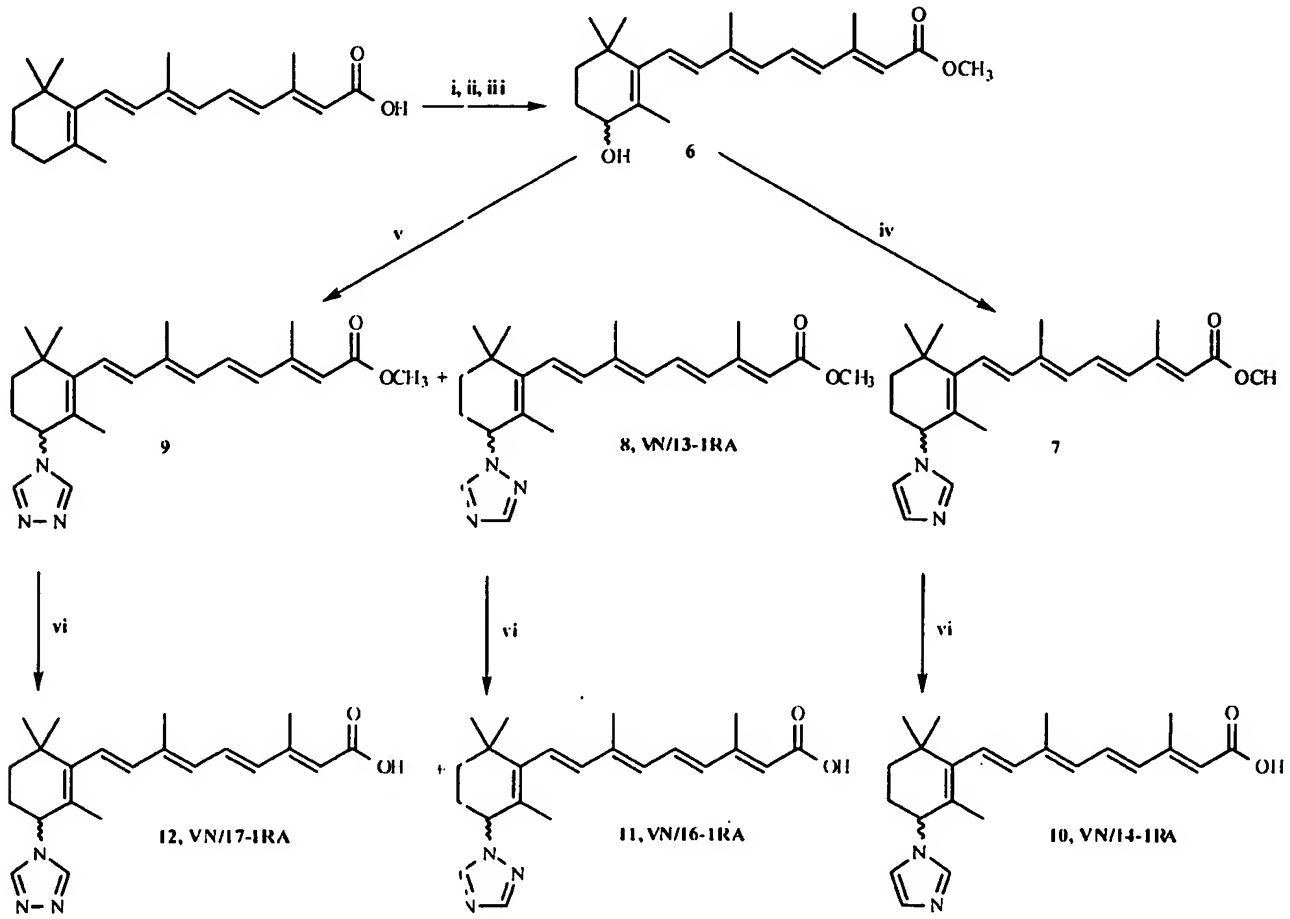
are related to its inhibition of ATRA metabolism and that the previously demonstrated inhibition of CYP17 (inhibition of androgen synthesis) is marginal *in vivo*.

Although a number of researcher have questioned liarozole's mechanism of action [56, 57], we are of the opinion that there is currently sufficient evidence (*vide supra*) to ascribe its mechanism of action to inhibition of ATRA metabolism. Although it has been suggested that its mechanism of action may also involve inhibition of aromatase, and of androgen biosynthesis: the fact that specific and potent aromatase inhibitors were without effects in clinical trials of patients with prostate cancer (PCa), and that androgen synthesis inhibitors are ineffective in androgen-independent prostate tumors makes a strong case for their irrelevance in this setting.

A large Phase III international study has very recently been completed comparing liarozole 300 mg twice daily with cyproterone acetate (CPA) 100 mg twice daily in a total of 321 patients with metastatic prostate cancer in relapse after first-line endocrine therapy [58]. The adjusted hazard ratio for survival was 0.74 in favor of liarozole ($P = 0.039$), indicating a 26 % lower risk of death than in patients treated with CPA. Liarozole was superior to CPA in terms of prostate-specific antigen (PSA) response, PSA progression, and survival, and was capable of maintaining patients' quality of life. The observed adverse events were relatively mild to moderate in nature. The results indicate that liarozole might be a possible treatment option for PCa following failure of first-line endocrine therapy.

Although most experiences of liarozole as an anti-cancer agent have been limited to prostate cancer, a few experiments with breast cancer have also been conducted. In cultured human breast cancer MCF-7 cells, liarozole potentiated the antiproliferative and differentiative effects of ATRA [59-62]. This enhancement of ATRA effects could be explained by the inhibition of enzymatic degradation of ATRA in these cells.. Liarozole has proven antitumor activity in steroid-insensitive TA3-mouse mammary carcinoma and in NUM-induced mammary carcinoma in rats [63].

Despite these encouraging preclinical and clinical results, the usefulness of liarozole therapy is considered limited due to adverse side effects that are attributed to its lack of CYP



Reagents and conditions: i, $TMSCHN_2$/benzene: MeOH, N_2, rt.; ii, Activated MnO_2/CH_2Cl_2, rt.; iii, $NaBH_4$/MeOH, rt.; iv, CDI/CH_3CN, rt.; v, CDT/CH_3CN, rt.; iv, 10% KOH/MeOH, Ar, reflux.

Scheme 3. Synthesis of 4-azolyl retinoids.

isozyme specificity and its moderate potency of ATRA 4-hydroxylase. Consequently, Janssen have since discontinued clinical development of liarozole [64].

NEW INHIBITORS OF ALL-TRANS-RETINOIC ACID CYTOCHROME P450 4-HYDROXYLASE

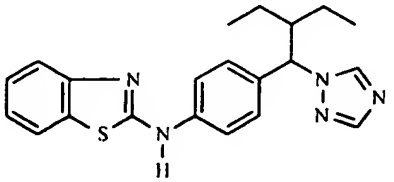
Azolyl Retinoids

The emerging role of RAMBAs as potential agents in the treatment of both hormone-dependent and hormone-independent cancers [10, 65, 66] has led to our interest in this area. Given the significance of azole grouping of many drugs which are P450 enzymes [67-69], we reasoned that introducing azole group at C-4 (the site of initial enzymatic hydroxylation) of ATRA should yield specific and potent inhibitors of ATRA 4-hydroxylase. Indeed, we very recently described the synthesis of a number of novel 4-azolyl ATRA derivatives, some of which are amongst the most potent inhibitors of this enzyme [70, 71]. The synthesis of these compounds is outlined in "Scheme (3)", with the key reaction being the near quantitative formation of the 4-azolyl retinoids (**7-9**) following the reaction of a lylic alcohol (**6**) with N, N'-carbonyldiimidazole (CDI) or N, N'-carbonylditriazole (CDT) [71, 72].

The azolyl ester, 4ξ-(1*H*-1,2,4-triazol-1-yl)methyl retinoate (**8, VN/13-1RA**), and the free acids, 4ξ-(1*H*-imidazol-1-yl)retinoic acid (**10, VN/14-1RA**), 4ξ-(1*H*-1,2,4-triazol-1-yl)retinoic acid (**11, VN/16-1RA**) and 4ξ-(4*H*-1,2,4-triazol-1-yl)retinoic acid (**12, VN/17-1RA**) exhibited highly potent inhibition of hamster liver microsomal RA 4-hydroxylase with IC_{50s} of 680, 100, 880 and 1600 nM, respectively, being 4 - 60 times more potent than liarozole (IC_{50} = 6000 nM). Under this assay conditions, keto had an IC_{50} value of 34000 nM [70, 71]. Preliminary antiproliferative activity of these novel azolyl RAMBAs (**7-12**) have been tested against a human breast cancer cell line, MCF-7 and two prostate cancer cell lines, the androgen dependent LNCaP and androgen independent PC-3. Each of these compounds significantly enhanced the antiproliferative action of ATRA on these cell lines, and they were more effective than liarozole[1]. Further studies with these novel azolyl RAMBAs, and the identification of other new compounds are in progress. World and USA patent applications to protect these new compounds have been filed.

(B)-N-[4-[2-ethyl-1-(1H-1,2,4-triazol-1-yl)butyl]phenyl]-2-benzothiazolamine, R115866

Researchers at Janssen Research Foundation (JFR) have recently reported on a novel 1H-triazole derivative, R115866, **13** ("Fig. (2)") that is a potent inhibitors of human CYP26A1 (IC_{50}, 4 nM), being 750 times as potent as liarozole (IC_{50}, 3 μM) [73]. However, details of its synthesis are yet to be reported. R115866 is highly selective for CYP26 as it exhibited mediocre inhibitory effects on aromatase, CYP17, CYP21I, CYP3A, and CYP2A1, respectively. *In vivo*, administration of R115866 (2.5 mg/kg, p.o) to rats induced significant and transient increase of endogenous ATRA levels in plasma, skin, fat, kidney, and testis. Consequently, the compound exerted retinoidal effects, for example, inhibition of vaginal keratinization in rats. Although these studies with R115866 seem to be focused on dermatological therapy [73, cited in 74], their potential as agents for the treatment of cancers are warranted. Because of the compound's high inhibitory potency and selectivity for CYP26A1, it should be considered less likely to produce unwanted side effects as those experienced with liarozle therapy.



13, R115866

Fig. (2). Structure of R115866.

R116010

Limited data on a novel RAMBA, R116010 (structure not available) have been presented at several scientific meetings in abstract forms by researchers at JFR[2]. R116010 is reported to be 100-fold more potent than liarozole, and selective for CYP26A versus other cytochrome P450-dependent metabolizing enzymes. Furthermore, R116010 exhibited strong antitumor effects against androgen-independent rat prostate adenocarcinoma R3327/PIF1 and also against estrogen-independent mouse TA3-Ha mammary tumors. These results suggest that R116010 is a suitable candidate for further development for the treatment of prostate and breast cancers.

CONCLUSION

Retinoid therapy is based on differetiation of premalignant and malignant cells with the potential of redirecting the cells towards their normal phenotype. However, exogenous retinoid therapy is yet to fulfil the expectations raised by *in vitro* and *in vivo* studies in cancer models in the clinics. Modulation of endogenous ATRA and possibly its natural sterioisomers with the use of new RAMBAs may present an addition cancer therapy strategy. It

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is clear from this mini review that only a few potent RAMBAs are currently available. We are presenting this review with the goal of stimulating interest in the area to enable more studies that may lead to new RAMBAs that would be directed towards their potential evaluation as therapeutics in oncology and dermatology.

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NOTE ADDED IN PROOF

J. Van Heusden and others have recently presented a detailed study that characterize R116010 (structure given) as a potent and selective inhibitor of all-*trans* retinoic acid metabolism, exhibiting antitumor (of murine estrogen-independent TA3-Ha mammary tumors) activity. (*Br. J. Cancer*, **2002**, *86*, 605-811).

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The Emerging Role of Retinoids and Retinoic Acid Metabolism Blocking Agents in the Treatment of Cancer

Wilson H. Miller, Jr., M.D., Ph.D.

Lady Davis Institute for Medical Research and SMBD Jewish General Hospital, Department of Oncology, McGill University, Montreal, Quebec, Canada.

BACKGROUND. Although significant advances have been made in the treatment of some malignancies, the prognosis of patients with metastatic tumors remains poor. Differentiating agents redirect cells toward their normal phenotype and therefore may reverse or suppress evolving malignant lesions or prevent cancer invasion. In addition, they offer a potential alternative to the classic cytostatic drugs.

METHODS. The purpose of this review was to examine the current and potential future roles of differentiating agents in the treatment of cancer.

RESULTS. Initial studies with differentiating agents focused on retinoid therapy. Although retinoids have shown some clinical success, their widespread use has been limited by resistance and, in the chemopreventive setting, toxicity. This has led to the synthesis of a number of new retinoids that currently are undergoing clinical investigation. A further approach to overcoming the drawbacks associated with exogenous retinoids has been to increase the levels of endogenous retinoic acid (RA) by inhibiting the cytochrome P450-mediated catabolism of RA using a novel class of agents known as retinoic acid metabolism blocking agents (RAMBAs). Liarozole, the first RAMBA to undergo clinical investigation, preferentially increases intratumor levels of endogenous RA resulting in antitumor activity.

CONCLUSIONS. Although studies using exogenous retinoids in this setting have not yet fulfilled their initial promise, studies with a growing set of synthetic retinoids are ongoing. Furthermore, modulation of endogenous retinoids may offer a significant new potential treatment for cancer. *Cancer* **1998;83:1471–82.**

KEYWORDS: retinoic acid, retinoic acid metabolism blocking agents (RAMBAs), liarozole, retinoids, cancer, differentiation.

During the last 30 years, research into cancer treatment has focused mainly on the use and development of cytotoxic agents. However, despite significant progress in the chemotherapy of some malignancies such as testicular carcinoma and lymphomas, the prognosis of patients with the most common invasive and metastatic tumors remains poor.[1] There is a clear need for new treatment approaches, which ultimately may be met by novel ideas coming from recent advances in understanding the underlying biology of cancer. Cancer cells show various degrees of differentiation, and there normally is an inverse relation between the degree of cell differentiation and the clinical aggressiveness of a cancer.[2]

However, certain chemicals (differentiating agents) are capable of redirecting the cells to the normal phenotype of morphologic maturation and loss of proliferative capacity. Consequently, differentiating

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Address for reprints: Wilson H. Miller, Jr., M.D., Ph.D., Lady Davis Institute for Medical Research and SMBD Jewish General Hospital, Department of Oncology, McGill University, 3755 Chemin de la Cote-Sainte-Catherine, Montreal, Quebec H3T 1E2, Canada.

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agents may reverse or suppress evolving lesions or prevent cancer invasion.[1,3–6] Differentiating agents thus offer an attractive potential alternative to conventional cytotoxic agents. One group, the retinoids, constitute a class of chemical compounds, including vitamin A and its synthetic and naturally occurring analogs, that have been the subject of extensive scientific and clinical investigations.[6–9] However, despite the synthesis and evaluation of thousands of retinoids over the past 20 years, clinical success remains limited. The majority of reviews of the effectiveness of retinoids in cancer treatment and prevention conclude that we require new agents that are more effective or, especially in the setting of chemoprevention, less toxic.[10–12] Research to date has concentrated on the use of exogenous retinoids in cancer. Although this research continues with new retinoid derivatives, an alternative approach to the treatment and prevention of cancer is the use of retinoic acid metabolism blocking agents (RAMBAs), which increase levels of endogenous retinoic acid (RA) within the tumor cells by blocking their metabolism. This approach presents several theoretic advantages.

Endogenous Retinoids

Vitamin A (retinol) is obtained from the diet as preformed retinoids (retinyl-esters) from animal sources and as provitamin carotenoids (including β-carotene) from plant sources. These are converted to retinol in the gut, absorbed, and stored in the liver as retinyl palmitate. All-*trans*-retinoic acid (tRA) (tretinoin), 13-*cis*-retinoic acid (13cRA) (isotretinoin), 9-*cis*-retinoic acid (9cRA), and retinal (vitamin A aldehyde) are naturally occurring retinol derivatives.

In the plasma, retinol and tRA are bound tightly to retinol-binding protein (RBP) and albumin, respectively.[13] Retinol is the major circulating retinoid in the human body and its plasma levels remain near 2 μmol/L under normal conditions.[13] In contrast, normal plasma tRA and 13cRA levels range from 4–14 nmol/L[14] and 3.7–6.3 nmol/L,[15] respectively. 9cRA also has been shown to occur naturally in vivo, although the levels found are lower than that of tRA.[16]

Retinoid Physiology

Vitamin A plays an important role in the maintenance of normal growth, vision, reproduction, and bone formation. Vitamin A deficiency results in night blindness (the earliest manifestation), inhibition of spermatogenesis, and potential teratogenesis. In animals, vitamin A deficiency has been associated with a higher incidence of cancer and increased susceptibility to chemical carcinogens.[9] A variety of retinoids inhibit the in vivo development of carcinogen-induced carcinoma of the bladder, breast, liver, lung, pancreas, prostate, and skin.[5,6,8,17]

tRA is very potent in promoting growth and controlling differentiation and maintenance of epithelial tissue in vitamin A-deficient animals. tRA is considered the active form of retinol in all tissues except the retina[9,18] in which retinal is essential, and is 10- to 100-fold more potent than retinol in various in vitro systems.[19] In vitro studies have suggested 9cRA may have a specific role in the regulation of apoptosis.[20,21]

A role for retinoids in the physiology of prostate carcinoma has been suggested by Pasquali et al.[22] In the study, the concentration of tRA was lower in prostate carcinoma tissue compared with normal prostate and benign prostate hyperplasia. The lower levels of tRA were believed to be due either to the rapid degradation of tRA associated with increased activity of dehydrogenase enzymes or increased amounts of cellular tRA binding protein. The low levels of tRA in prostate carcinoma tissue may create a more permissive environment for the tissue to undergo cellular transformation or tumorigenesis. Increasing the endogenous levels of RA in prostate carcinoma cells by inhibiting its metabolism could result in differentiation of these tumor cells toward more normal behavior.

Nuclear Retinoic Acid Receptors

At the molecular level, the biologic effects of retinoids are modulated through nuclear receptors. Six nuclear retinoid receptors (RAR and RXR, both with α, β, and γ subtypes) that are members of the steroid-thyroid superfamily of nuclear receptors have been identified.[23–27] The three RARs have substantial homology and become transcriptionally active by binding with tRA or 9cRA.[28] Early studies suggested that the binding affinity of RARβ for tRA was tenfold that of RARα[29] but later studies demonstrated similar tRA binding affinities for both receptors.[30] RARα and RARβ show poor binding for retinol and retinal and at least a fivefold lower binding affinity for 13cRA than tRA or 9cRA.[30] In contrast, RXRs bind with 9cRA but not with 13cRA or tRA.[16,31]

The activated nuclear receptors control the expression of genes that mediate retinoid effects, including regulation of cell differentiation, growth, and induction of apoptosis.[9] Because RARs, RXRs and other members of the superfamily of nuclear receptors form heterodimers to induce transcription of a variety of DNA response elements,[32,33] the pleiotropic action of retinoids may result from specific heterodimers with distinct transcriptional attributes.[34] Additional complexity is provided by the recent discovery that a number of ligand-regulated transcriptional intermediates

play critical roles in transcription induced by multiple nuclear steroid hormone receptor family members.[33] They directly stimulate or inhibit, often in a ligand-dependent fashion, transcription from DNA bound receptors, perhaps by influencing the linkage between the promoter complex and the basal transcription factors.[33,35]

Several studies have correlated the sensitivity of malignant cells to retinoids with the presence or level of expression of nuclear retinoid receptors. An inactivating mutation of the RARα gene was shown to be present in tRA-resistant leukemic cells; when the normal RARα was reexpressed in the cells, sensitivity was restored.[36] The RARα gene also has been shown to be located at the chromosomal breakpoint associated with acute promyelocytic leukemia (APL), a malignancy particularly sensitive to retinoids.[37–40] There is evidence that RAR expression is higher in retinoid-sensitive human breast carcinoma cells compared with retinoid-resistant cells.[41–42] The response to RA of squamous premalignant and malignant cells has been associated with the expression of RARβ.[6] However, other RARs can substitute for the mutated RARα in the leukemia model mentioned earlier,[43] and the gene (PML) fused to RARα in APL may play an equally important role in the malignant phenotype.[44] In addition, breast carcinoma cells have been shown to regain responsiveness to retinoids without changes in RAR expression[45] and modulators of retinoid metabolism or novel retinoids may inhibit tumor cell growth without obvious interaction with known retinoid receptors.[46] Clearly, more research is needed in this area.

Cytoplasmic Retinoic Acid Binding Proteins

tRA appears to enter cells by simple diffusion. Once within the cell, distinct intracellular cytoplasmic binding proteins have been identified for tRA (cellular retinoic acid binding proteins I and II [CRABPI and CRABPII])[13,47] and retinol (cellular RBPs [CRBPI and CRBPII]).[7,47–49] The functions of CRBPs and CRABPs are not well defined; it initially was believed that they were responsible for intracellular retinoid transport,[50] but it now appears that they also may regulate free concentrations of retinol and tRA and play a role in retinoid metabolism.[47,49,51]

Boylan and Gudas reported decreased in vitro responses to tRA in cell lines with overexpressed cellular binding proteins.[52] A direct role for CRABP in tRA metabolism also has been reported; metabolism of tRA bound to CRABP is 7-fold more efficient than that of free tRA.[53] tRA has been shown to increase CRABPII expression in both normal and leukemic hematopoietic cells, and this induction may contribute to the

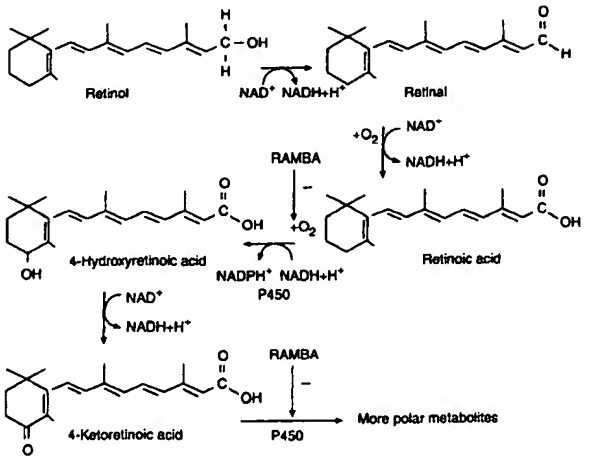

FIGURE 1. Metabolic pathway for retinol and retinoic acid. P450 = mediated by cytochrome P450 enzyme. RAMBA: retinoic acid metabolism blocking agents.

increased catabolism and subsequent clinical resistance to tRA observed in patients with APL given continuous oral dosing of tRA.[54,55] These studies have led investigators to search for inhibitors of retinoid metabolism or retinoids that do not bind CRABP for use in refractory APL. Both 9cRA and 13cRA can maintain more stable plasma levels, in part because of reduced binding to CRABP, although neither drug has been particularly successful in this clinical setting, suggesting multiple possible mechanisms of resistance.[39,56]

Conversely, there is evidence in some systems that binding to CRABPs may be associated with increased biologic effects. CRABP has a much higher binding affinity for tRA and synthetic retinoids that have high potency (≥ that of tRA) than for retinol, retinal, and a variety of ineffective compounds.[47] Some teratocarcinoma cell lines that do not differentiate in response to tRA have markedly reduced CRABP levels,[57,58] although introduction of the CRABP gene into the cells does not restore sensitivity.[52] A correlation between tRA metabolism in tumor cells and the sensitivity of these cells to differentiation therapy, which may be mediated by CRABP levels, recently has been reported in human and murine cell lines.[59]

Retinoid Metabolism

In humans, retinol is oxidized to retinal, which is in turn oxidized to tRA. tRA then undergoes cytochrome P450-dependent hydroxylation followed by oxidation to 4-oxo-metabolites[51,60–62](Fig. 1) that are conjugated with glucuronic acid and excreted in the bile.[63]

As discussed earlier, the pharmacodynamic effects of tRA may be attenuated by its rapid rate of

TABLE 1
Biologic Effects of Retinoids That Are Relevant to the Prevention and Treatment of Cancer

Biologic effects	References
Induction of cytodifferentiation	[8,9,17, 71–78]
Inhibition of cell proliferation	[7,9,17,79]
Stimulation of host immune response	[7,17,80]
Augmentation of cell-mediated cytotoxicity	[11,17]
Inhibition of oncogene expression	[7,17,77,80,81]
Apoptosis	[17]
Suppression of transformed phenotype	[7,17,76,79,81]
Inhibition of angiogenesis	[7,80]
Modulation of cell migration, adhesion, and invasion	[82–84]
Reduction of collagenase, stromelysin, and plasminogen activator levels	[85]
Modulation of cell surface glycoconjugate levels	[77,86,87]
Antioxidant activity and free radical deactivation	[17]
Stimulation of epidermal growth factor, transforming growth factor-β, and tumor necrosis factor activities	[17]

metabolism. The ability of cytochrome P450 inhibitors (such as imidazoles) to suppress tRA metabolism[64,65] and delay tRA plasma clearance has been demonstrated in animals[65,66] and humans[67,68] administered concomitant tRA and cytochrome P450 inhibitors. However, no attempt to use these restored plasma tRA levels to restore sensitivity to the drug in vivo has been reported.

Recently, it has been suggested that the low levels of endogenous RA observed in oral premalignant lesions could be due to increased metabolism of RA.[7] The use of RAMBAs to overcome this effect could result in therapeutic benefits.

Anticancer Effects of Retinoids

The importance of retinoids in cancer dates from the 1920s, when epithelial changes including hyperkeratosis, squamous metaplasia,[69] and carcinoma of the stomach[70] were observed in vitamin A-deficient animals. It is now known that retinoids exert numerous biologic effects that are germane to carcinogenesis, metastasis, and the chemoprevention of cancer (Table 1).[7–9,11,17,71–87] Our understanding of the intracellular cascade of events initiated by tRA binding with retinoid receptors is elementary. However, it is evident that tRA exerts antitumor activity by the promotion of cell differentiation, apoptosis, and the inhibition of cell proliferation.

Exogenous Retinoids in Cancer

Initial attempts to administer pharmacologically active doses of first-generation retinoids (such as tRA [tretinoin] and 13cRA [isotretinoin]) were limited both by toxicity and, in the case of tRA, poor pharmacodynamics. Therefore attention turned to the development of synthetic retinoids with improved therapeutic indices.[88] Modification of the basic retinoid molecule has produced > 2500 retinoids in the past 25 years,[10] including second-generation and third-generation compounds. Continued research also has discovered new naturally occurring retinoids, including 9cRA and, more recently, a variety of 4-oxoretinoids.[89,90]

Preclinical and clinical studies have shown retinoids to possess activity in the prevention and treatment of cancer. The successes achieved with tRA, cRA, etretinate, fenretinide, and newer retinoids already have been reviewed extensively.[4,6,10,12,91] Retinoids may mediate multiple anticancer mechanisms, including induction of cell differentiation, inhibition of proliferation by cell cycle arrest, and induction of apoptosis.[9] tRA induces differentiation and proliferation in various murine and human malignant cell lines in vitro.[7,76–78,92,93] 9cRA has been found to have comparable effects in the majority of models tested.[39,45,94]

Clinical Studies: Treatment

Hematologic malignancies

Differentiation-induced complete remissions have been achieved with tRA in patients with APL.[58,95–98] Although tRA initially appeared to be a better inducer of complete remission in APL than 13cRA,[56] recent studies suggest 9cRA is at least as effective[39] and that RAR specific ligands may be even better.[99] Although the initial response to exogenous tRA in patients with APL is high, the duration of complete remission is short (median, < 6 months) and few patients can be maintained in continuous complete remission on tRA monotherapy.[100–102] Therefore APL patients who achieve complete remission with tRA require intensive postremission chemotherapy to maintain the remission. The combination of tRA and chemotherapy has become the current standard treatment for APL, achieves complete remission rates of approximately 90%, and has been shown in randomized trials to improve long term survival.[91] Unfortunately, other subtypes of acute myelocytic leukemia do not respond to the retinoids studied to date.[103] However, there is in vivo, as well as in vitro, evidence that some lymphomas may be responsive to retinoids.[104]

Nonhematologic malignancies

Although retinoids have not produced the dramatic results observed with APL in other advanced malignancies, promising results have been obtained in trials combining tRA or 13cRA with other agents such as interferon-α-2a in patients with nonhematologic malignancies such as squamous cell carcinoma[105,106] and

metastatic renal cell carcinoma. Because Kaposi's sarcoma cells are sensitive to RA in vitro, a variety of topical and systemic retinoids recently have been used in clinical trials against Kaposi's sarcoma.

Clinical Studies: Chemoprevention

Primary

Early results from epidemiologic studies suggested an inverse relation between dietary intake of vitamin A or β-carotene and the incidence of cancer.[70] More recent case-control epidemiologic studies comparing high with low vitamin A intake showed an overall risk reduction for laryngeal, lung, esophageal, and tongue carcinoma of 54%, 73%, 44%, and 59%, respectively,[107] and a vitamin A-deficient diet yielded relative risks of 1.1 to 2.6 for solid tumors in general and 1.5 to 2.0 for lung carcinoma.[108] However, large scale studies in Finland,[109] the U. S.[110,111] and a multinational study conducted in Europe, Japan, and the U. S.[112] have concluded that one specific compound, β-carotene, does not have a beneficial primary chemoprotective effect. However, in retrospect, the rationale for selection of this particular carotenoid isomer is not well justified. These large trials simply may show that β-carotene, which is one of many related compounds isolated from fruits and vegetables shown to lower cancer risk, is not the active agent of risk reduction. Whether these results can be generalized to other retinoid-related compounds, or even other carotenoids, is not known.

Secondary

Overall, retinoids have shown significant activity in the reversal of cervical, oral, and skin premalignancies, and in the prevention of head and neck, lung, and skin primary or second primary tumors, although further research clearly is needed.[4,6,10–12,111–115] Several large, randomized placebo-controlled trials involving retinoids currently are ongoing.[114, 116] For example, the European Organization for Research and Treatment of Cancer EUROSCAN Trial (which commenced in 1988), is investigating the effect of retinol palmitate (300,000 IU) on the prevention of second primary tumors in patients with head and neck carcinoma.[116]

Unfortunately, the chronic toxicity of currently available agents precludes the conduct of primary chemoprevention trials in healthy populations at increased risk of developing cancer. Newer retinoids with potentially lower toxicities currently are being evaluated and include 4-hydroxyphenyl retinamide (fenretinide), which concentrates in mammary tissues and has been shown to prevent mammary tumors in rats.[46,117] An ongoing trial in Milan is evaluating fenretinide, 200 mg/day for 1 year (vs. no treatment), in the prevention of secondary tumors in patients with surgically resected oral leukoplakias.[114] A second study also is underway evaluating fenretinide, 200 mg for 5 years (vs. no treatment), in the chemoprevention of breast carcinoma. The aim of the study, which has enrolled 2972 randomized patients, is to evaluate the efficacy of the agent in reducing contralateral breast primary tumors. Preliminary data from the trial, which was initiated in 1987, indicate no difference between the two groups.[118]

Drawbacks of Retinoid Therapy

Adverse effects

Chronic administration of high doses of vitamin A produces hypervitaminosis A, a toxicity characterized by anorexia, weight loss, fever, hepatosplenomegaly, skin and mucous membrane changes, alopecia, cheilitis (cracking and bleeding lips), bone and joint pain, hyperostoses, thrombocytopenia, and elevated cerebral fluid pressure. The natural retinoids 9cRA, 13cRA, or tRA produce similar adverse effects; however, a more diverse adverse events profile is observed with synthetic retinoids that may not exert the same biologic properties as vitamin A (Table 2).

The majority of these side effects are reversible after discontinuation of treatment but bone toxicities and some visual disturbances may persist. Most important, profound teratogenic effects from retinoids limit their use in women of childbearing age. This is complicated further by the long tissue half-life of some synthetic retinoids. Of note, a study by Besa et al. in transfusion-dependent patients with myelodysplastic syndrome reported that α-tocopherol significantly reduced the severe skin and constitutional toxicities observed with 13cRA treatment, allowing long term treatment with the retinoid.[119]

A further side effect has only been documented in patients receiving retinoids for APL. Approximately 25–30% of patients receiving systemic tRA for the treatment of APL experience "retinoic acid syndrome," comprising leukocytosis, thrombosis, fever, respiratory distress, pulmonary infiltrates, pleural effusions, and weight gain.[120] Although early intervention with corticosteroids can prevent progression of the syndrome, several patients have died of multiple organ failure.[102] The early use of chemotherapy also may benefit some patients. This syndrome also has been observed in patients treated with 9cRA,[39] further suggesting it is specific for APL and not the particular retinoid used. Whether there will be additional life-threatening retinoid side effects that are disease specific remains to be determined.

TABLE 2
Adverse Events Observed with Commonly Used Exogenous Retinoids

Toxicity	tRA	13cRA	9cRA	Etretinate	Fenretinide
CNS					
Headache	✓	✓	✓	x	x
Skin and mucous membranes					
Cheilitis	✓	✓	✓	✓	x
Itching	✓	✓	✓	✓	✓
Desquamation	✓	✓	✓	✓	✓
Alopecia	x	✓	?	✓	x
Dryness	✓	✓	✓	✓	✓
Ocular					
Dry eyes/conjunctivitis	✓	✓	✓	✓	✓
Night blindness	x	x	?	✓	✓
Lipid profile abnormalities	✓	✓	✓	✓	x
Hepatotoxicity	✓	✓	✓	✓	x

tRA: all-*trans*-retinoic acid; 13cRA: 13-*cis*-retinoic acid; 9cRA: 9-*cis*-retinoic acid; CNS: central nervous system; ✓: event observed; x: event not observed; ?: uncertain.

Retinoid Resistance

As discussed earlier, the complete remissions achieved using tRA monotherapy in APL are brief, and patients who recur during tRA treatment cannot be reinduced into complete remission with tRA. This is observed even when the dose is doubled[98,121] and despite no apparent increased resistance to conventional chemotherapy.[95–98,122]

Although the mechanism for resistance is unclear, it may be due to decreasing plasma levels resulting from a consistent acceleration of tRA metabolism.[8,121] In one study of patients receiving tRA for APL, the decrease in plasma drug levels corresponded with clinical recurrence. Although these patients clinically were resistant to further tRA treatment, their leukemic cells remained sensitive to the cytodifferentiating effects of the drug in vitro.[121] This suggests that retinoid resistance and recurrence from tRA-induced remission during prolonged administration may reflect an inability to maintain drug levels that are adequate to induce cytodifferentiation.

The ability of tRA to act as an autoinducer of catabolism poses a clinical problem if tRA levels cannot be sustained adequately to produce the desired therapeutic response. Although transient achievement of therapeutic levels may induce terminal differentiation of APL cells, this problem would be particularly limiting in the chemoprevention setting. In addition, administration of exogenous retinoids may result in lower absorption and/or storage of retinol; in one study, the mean plasma retinol levels decreased by 60% within 14 days of commencing fenretinide therapy.[123] Because an adequate amount of retinal (which is derived from retinol) must be available to ensure normal functioning of the retina, this interference results in adverse effects on vision.[123]

One approach to this problem has been to substitute 9cRA for tRA in APL. A pharmacokinetic study showed relatively little change in the metabolism of 9cRA after several weeks of dosing.[39] In spite of that, 9cRA did not reverse clinically acquired retinoid resistance.[39] It is not known whether there was an undetected effect of continuous dosing on destruction of important metabolites of 9cRA or whether, in this study, new molecular abnormalities were the basis of clinical resistance.

Recent studies of retinoid-resistant cell lines suggest novel molecular mechanisms of resistance may play at least as important a role as pharmacologic mechanisms. Structural mutations in RARα have been associated with the development of retinoid resistance in several cell lines.[43] In vitro, the dominant negative PML/RARα oncoprotein of APL is a direct target of retinoid action in RA-sensitive APL cells but not in APL cell lines derived for RA resistance.[124,125] Studies of retinoid-induced gene expression and function of the PML/RARα protein in these resistant cells suggest no altered RA pharmacology, but rather suggest that the appearance of a further mutation in the PML/RARα molecule selectively blocks the induction of differentiation-associated pathways by RA.[125, 126] Indeed, similar mutations were found in cells from patients with APL resistant to retinoids.[127] Another report links RA resistance to altered associations of PML/RARα with nuclear corepressor molecules that link gene transcription and chromatin structure.[128] Thus, there may be multiple mechanisms of retinoid resistance in vitro and in vivo, leading to new research directions.

Retinoic Acid Metabolism Blocking Agents

Preclinical and clinical studies provide substantial evidence for the therapeutic use of retinoids in cancer prevention and treatment. Although exogenous retinoids have demonstrated significant activity against some cancers, the doses required are associated with acute and chronic toxicities that may necessitate dose reductions, drug holidays, or treatment discontinuation. Furthermore, the use of exogenous tRA is hampered by the drawback of induction of retinoid metabolism.[102] The ideal retinoid should have minimal toxicity and no stimulatory effect on the cytochrome P450 system. As yet, no such agent fulfills these criteria.

A new approach to this problem is based on the occurrence of endogenous tRA,[14] 9cRA,[16,3] and 4-oxo-retinoids.[89,90] Retinoic acid metabolism blocking agents (RAMBAs) have been developed that inhibit the cytochrome P450 mediated catabolism of RA (4-hydroxylation of RA), thereby increasing tissue and plasma levels of endogenous RA, resulting in differentiation of cells.[129] The imidazole derivative liarozole, the first member of this class of RAMBA compounds, has shown antitumor properties.[130]

Effect on Endogenous RA Levels

A recent analysis of the oxidative catabolism of tRA in homogenates of rat liver and rat Dunning R3327G prostate tumors demonstrated that tRA metabolism was inhibited in a concentration-dependent manner by liarozole.[131] In in vivo studies using rats, oral liarozole at a dose of 5 or 20 mg/kg increased endogenous plasma tRA levels from < 0.5 ng/mL to 1.4 and 2.9 ng/mL, respectively.[66] Smets et al. reported that liarozole increased plasma and tumor RA levels in the Dunning AT-6sq androgen-independent prostate carcinoma model in a dose-dependent manner.[132] RA levels were increased preferentially in the tumor (sixfold increase) with levels increasing threefold in plasma. In further preclinical studies, liarozole prolonged the $t_{1/2\beta}$ of exogenously administered tRA.[133] The $t_{1/2\beta}$ of exogenously administered 9cRA and 4-keto RA similarly was increased, suggesting that multiple natural retinoids may be affected by liarozole.[133,134] To my knowledge no studies have reported that liarozole affects absorption or storage of retinol.

Effects of Liarozole on Proliferation and Differentiation of Tumor Cells

A number of studies have shown that liarozole has no direct in vitro antitumoral effects, although it inhibits RA metabolism. This inhibition can, in turn, augment the antitumor activity of retinoids. In two studies, liarozole inhibited the metabolism of tRA and thereby enhanced its antiproliferative effect in MCF-7 human breast carcinoma cells.[135–137] Similar results were obtained in mouse 10T1/2 embryonal cell lines; liarozole potentiated 1000-fold the ability of low concentrations of tRA to inhibit carcinogen-induced neoplastic transformation and protected tRA from catabolism over a 48-hour period.[138] A study in human glioblastoma cells also showed that liarozole enhanced the antiproliferative effects of tRA as measured by ^{3}H-thymidine incorporation,[139] whereas a study by Elder et al.[140] reported that in human skin fibroblasts, liarozole significantly increased fibroblast CRABPII mRNA levels (a measure of retinoid bioactivity) and potentiated the effects of retinol by 1.5-fold at concentrations at which liarozole alone had no effect.

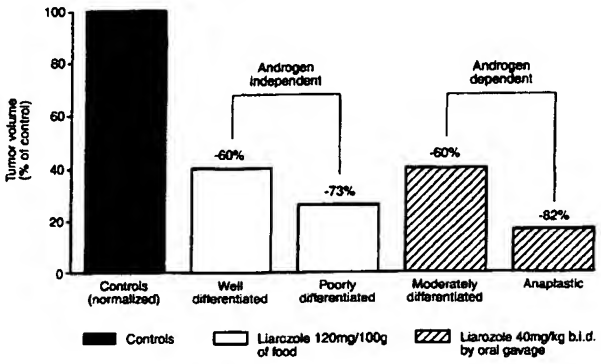

FIGURE 2. Effect of liarozole on androgen-dependent and androgen-independent Dunning prostate tumors in rats. Liarozole was administered as a dietary admixture in rats implanted with well differentiated, androgen-dependent Dunning H tumor or by oral gavage twice daily in rats implanted subcutaneously with androgen-independent (AT-sq) tumors. Control animals underwent castration or were treated with vehicle. Tumor volume was measured at the end of treatment. Based on information in Dijkman A, Van Moorsselaar RJA, Van Ginckel R, Van Stratum P, Wouters W, Debruyne FM, et al. Antitumoral effects of liarozole in androgen-dependent and -independent R3327 Dunning prostate adenocarcinomas. *J Urol* 1994;151:217–22.

Antitumor Effects of Liarozole In vivo

Although minimally toxic to tumor cells when given alone in vitro, liarozole alone has significant activity against tumors in vivo. Liarozole reduced tumor growth in rat models of androgen-dependent (G and H) and androgen-independent (PIF-1 and AT-6) prostate adenocarcinomas (Fig. 2).[141, 142] In the Dunning AT-6sq androgen-independent model, liarozole at a dose of 30 mg/kg significantly reduced tumor weight and induced accumulation of endogenous tRA tumor concentrations, whereas the differentiation status (measured by the cytokeratin profile of the carcinoma) shifted from a keratinizing toward a nonkeratinizing

squamous carcinoma.[130] Liarozole also inhibited subcutaneous and bone metastasis tumor growth of the androgen-independent PC-3ML-B2 human prostate carcinoma in SCID mice.[143] Overall, these antitumoral properties correlate with decreased endogenous retinoid metabolism, leading to an increase of tRA accumulation within the tumor cell.

Liarozole and Chemoprevention

The chemopreventive activity of liarozole has been investigated in rat prostate carcinoma induced by N-methyl-N-nitrosourea (MNU) followed by chronic exposure to testosterone. Liarozole was administered 1 week prior to MNU. Although liarozole-treated animals experienced similar incidence rates of microscopically detected carcinoma compared with controls, incidence rates of macroscopic carcinoma of all accessory sex glands, carcinoma of the dorsolateral prostate, and macroscopic carcinoma of the anterior prostate were reduced significantly compared with control groups.[144] Therefore liarozole inhibited the induction of prostate carcinoma (mainly at the progression stage) and suppressed the transition from microscopic or in situ lesions to macroscopic carcinoma.

Clinical Studies

The effect of liarozole on tRA catabolism was studied in a group of patients with solid tumors.[67] On Days 2 and 29 single doses of liarozole (75–300 mg) were given 1 hour before the administration of tRA. Liarozole significantly ($P = 0.004$) attenuated the decrease in the plasma tRA area under the curve.

Liarozole initially was investigated in patients with advanced prostate carcinoma who had failed hormone therapy. Results of 5 Phase I/II clinical studies in patients with hormone-resistant prostate carcinoma indicated that liarozole was associated with an objective response rate of 20%, an overall prostate specific antigen (PSA) response rate of 32%, and a good subjective response.[145] A recent multicenter, randomized, Phase III study comparing liarozole with the antiandrogen cyproterone acetate (CPA) in 321 patients with advanced prostate carcinoma who failed androgen ablation therapy reported that liarozole was superior to CPA with regard to overall survival (when adjusted for baseline imbalances), PSA response, and time to PSA progression.[146] However, more definitive trials may be needed.

Conclusions

The relative lack of clinical success with conventional anticancer agents may be due in part to the traditional concept of cancer being a biologic state rather than a dynamic process.[1] Redefining cancer as a dynamic disease commencing with carcinogenesis introduces the possibility of chemoprevention. Retinoids offer the promise of a therapeutic option based on differentiation of premalignant as well as malignant cells. Enormous advances have been made in the scientific and clinical studies of retinoids over the past decade, and further interesting developments are expected in the future. Although exogenous retinoids have not yet fulfilled hopes raised by their antineoplastic activity in vitro, studies with a growing set of synthetic retinoids are ongoing. Modulation of endogenous retinoids may offer an additional approach. The possibility of combining other anticancer drugs with exogenous retinoids or modulation of endogenous retinoids may offer a real opportunity to advance our ability to treat or prevent human cancer effectively.

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141. Van Ginckel R, De Coster R, Wouters W, Vanherck W, van der Veer R, Goeminne N, et al. Antitumoral effects of R 75251 on the growth of transplantable R3327 prostatic adenocarcinoma in rats. *Prostate* 1990;16:313–23.
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145. Denis, L. Liarozole-fumarate (LIA), a novel antitumoral drug: clinical update. Presented at Symposium on Recent Advances in Diagnosis and Treatment of Prostate Cancer, Quebec City, Quebec, Canada, September 21–23, 1995:45.
146. Debruyne JM, Murray R, Fradet Y, Johanssen JE, Tyrell C, Boccardo F, et al. Liarozole, a novel treatment approach for advanced prostate cancer: results of a large randomized trial versus cyproterone acetate. *Urology*. In press.

Curriculum Vitae

JAYASREE VASUDEVAN

Education

- Doctor of Philosophy in Chemistry (December 1994)

  Indian Institute of Chemical Technology, Hyderabad, India
  Thesis: Synthesis of Some Biologically Active Compounds
  Advisor: Dr. A.V. Rama Rao

- Master of Science (May 1989), 1st Class Honors

Organic Chemistry
Osmania University, Hyderabad, India

- Bachelor of Science (June 1987), 1st Class Honors

  Mathematics, Physics & Chemistry
  Osmania University, Hyderabad, India

Work Experience

- Primary CMC contact for interface with the FDA for both drug substance and drug product at the pre-IND and Phase 1/2a IND stage
- Preparation of pre-IND briefing packages and CMC sections for Phase 1/2a IND and IMPD
- Management of API and drug product manufacturing CROs for Phase 1/2a IND enabling toxicology studies and early phase clinical studies
- Member, Research Management Committee, Allergan-Cytochroma research collaboration (May 1999-May 2004) that led to identification of a P450RAI inhibitor lead candidate for dermatological disease treatment
- Senior Research Scientist/CMC at Vitae Pharmaceuticals (May 2004-present)
- Principal Scientist in Retinoid Research at Allergan, Inc. (August 2002-April 2004)
- Senior Scientist in Retinoid Research at Allergan, Inc. (November 1999-July 2002)
- Scientist in Retinoid Research at Allergan, Inc. (September 1997-November1999)
- Post-Doctoral Fellow (December 1994 - December 1996)

Department of Chemistry,
Rutgers, The State University of New Jersey
Supervisor: Professor Spencer Knapp

Academic Honors and Awards

- **Junior Research Fellowship**,Council of Scientific and Industrial Research (CSIR), India, 1989-1991.
- **Senior Research Fellowship**, Council of Scientific and Industrial Research (CSIR), India, 1991-1994.
- **Certificate of Merit**, Council of Scientific and Industrial Research, 1989. Awarded for qualifying in top 5% in the CSIR-UGC examination.
- **Gold Medal,** Osmania University, 1989. Awarded for the most outstanding scholastic achievement at the Graduate level.
- **Gold Medal**, Osmania University, 1987. Awarded for the most outstanding scholastic achievement at the Undergraduate level.

Research Experience

Organic Chemistry:

During my doctoral program, I was involved in the total synthesis of a natural product, an anti-HIV marine alkaloid Batzelladine A. Towards this end, I synthesised the tricyclic guanidine segment of Batzelladine A. During my post doctoral training, I was involved in synthesizing models for the photosynthetic reaction center of bacterial photosystems. Towards this goal, I synthesized a series of self-coordinating Zn(II) porphyrin dimers with a Zn-ligating pyridine tether, and conducted structure-based spectroscopic comparisons depending on the extent and direction of the porphyrin overlap.

Drug Discovery:

I worked as a medicinal chemist in drug discovery with retinoids mainly in the area of RXR agonists for oncology and diabetes and P450RAI inhibitors for the treatment of photodamage, acne and psoriasis at Allergan. The research effort on P450RAI inhibitors was conducted in collaboration with Cytochroma. I served as a member of the joint research committee and was involved as the primary medicinal chemist designing and making P450RAI inhibitors and managing up 2-5 MS level chemists during this period. I was also responsible for managing, interpreting and presenting the data generated at Cytochroma.

Drug Development and Regulatory Writing:

I was recently involved in the development of a P450RAI inhibitor, an RXR agonist and an RAR subtype selective agonist in the areas of dermatology and oncology as it pertains to scale up and procurement of drug substances and drug products and coordination of chemistry related development activities at Vitae. I was also involved in the preparation of CMC sections for two Phase 1/2a INDs.

Research Patents/ Patent applications

1. **Jayasree Vasudevan, Rong Yang, Liming Wang, Xiaoxia Liu, Kwok-Yin Tsang, Ling Li, Janet Takeuchi, Thong Vu, Richard Beard, Smita Bhat, Vidyasagar Vuligonda, Roshantha A. S. Chandraratna.** "Methods for treating retinoid responsive disorders

using selective inhibitors of CYP26A and CYP26B", ***U.S. Patent Application***, 20050187298, August 25, 2005.

2. **Jayasree Vasudevan, Liming Wang, Xiaoxia Liu, Kwok-Yin Tsang, Ling Li, Janet Takeuchi, Thong Vu, Richard Beard, Smita Bhat, Vidyasagar Vuligonda, Roshantha A. S. Chandraratna.** "Compounds having selective cytochrome P450RAI-1 or selective cytochrome P450RAI-2 inhibitory activity and methods of obtaining the same", ***U.S. Patent Application***, 20050176689, August 11, 2005.

3. **Yang-Dar Yuan, Jayasree Vasudevan, Scott Thacher, Roshantha A. Chandraratna.** "Compositions and methods using compounds having cytochrome P450RAI inhibitory activity co-administered with vitamin A", ***U.S. Patent Application***, 20040077721, April 22, 2004.

4. **Richard L. Beard, Tien L. Duong, Janet A. Takeuchi, Ling Li, Kwok-Yin Tsang, Xiaoxia Liu, Jayasree Vasudevan, Liming Wang, Santosh Sinha, Haiqing Yuan and Roshantha A. S. Chandraratna**. "7-[(7-Alkoxy)-chrom-3-en-6-yl]-heptatrienoic acid and 7-[(3-alkoxy)-5,6-dihydronaphthalen-2-yl]-heptatrienoic acid derivatives having serum glucose reducing activity", ***U.S. Patent***, 6,887,896, May 3, 2005.

5. **Jayasree Vasudevan, Alan Johnson, Liming Wang, Dehua Huang and Roshantha A. S. Chandraratna**. "Methods for identifying inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,855,512, Feb. 15, 2005.

6. **Jayasree Vasudevan, Richard L. Beard, Haiqing Yuan and Roshantha A. S. Chandraratna**. "3,5-Di-Iso-Propyl-Heptatrienoic Acid Derivatives Having Serum Glucose Reducing Activity ", ***U.S. Patent***, 6,759,546, July 6, 2004.

7. **Jayasree Vasudevan, Liming Wang, Yang-Dar Yuan, Xiaoxia Liu, Kwok-Yin Tsang and Roshantha A. S. Chandraratna**. "4-[(8-Ethynyl, 8-vinyl or 8-ethynyl-methyl)-6-chromanoyl]-benzoic acid and 2-[4-[(8-ethynyl, 8-vinyl or 8-ethynyl-methyl)-6-chromanoyl]-phenyl]-acetic acid, their esters and salts having cytochrome P450RAI inhibitory activity", ***U.S. Patent***, 6,740,676, May 25, 2004.

8. **Jayasree Vasudevan, Dehua Huang, Vidyasagar Vuligonda and Roshantha A. S. Chandraratna**. "Dihydrobenzofuran and dihydrobenzothiophene 2,4-pentadienoic acid derivatives having selective activity for retinoid X (RXR) receptors", ***U.S. Patent***, 6,720,423, April 13, 2004.

9. **Jayasree Vasudevan, Alan Johnson, Liming Wang, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,713,647, March 30, 2004.

10. **Jayasree Vasudevan, Alan Johnson, Liming Wang and Roshantha A. S. Chandraratna**. "1-Imidazolyl substituted tetrahydronaphthalene derivatives as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,603,019, August 5, 2003.

11. **Jayasree Vasudevan, Alan Johnson, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,531,599, March 11, 2003.

12. **Jayasree Vasudevan, Alan Johnson, Liming Wang, Dehua Huang and Roshantha A. S. Chandraratna**. "Methods of providing and using compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,495,552, December 17, 2002.

13. **Vidyasagar Vuligonda, Kwok Yin Tsang, Jayasree Vasudevan and Roshantha A. S. Chandraratna**. "2,4-Pentadienoic acid derivatives having selective activity for retinoid X (RXR) receptors". ***U.S. Patent***, 6,403,638, June 11, 2002.

14. **Jayasree Vasudevan, Alan Johnson, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent,*** 6,399,774, June 4, 2002.

15. **Jayasree Vasudevan, Alan Johnson, Liming Wang, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent,*** 6,387,951, May 14, 2002.

16. **Jayasree Vasudevan, Alan Johnson, Liming Wang, Dehua Huang and Roshantha A. S. Chandraratna**. "Methods of providing and using compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,387,982, May 14, 2002.

17. **Jayasree Vasudevan, Alan Johnson, Liming Wang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent,*** 6,380,256, April 30, 2002.

18. **Alan Johnson, Jayasree Vasudevan, Liming Wang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent,*** 6,369,261, April 9, 2002.

19. **Jayasree Vasudevan, Alan Johnson, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent,*** 6,369,225, April 9, 2002.

20. **Jayasree Vasudevan, Alan Johnson, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,359,135, March 19, 2002.

21. **Jayasree Vasudevan, Alan Johnson, Liming Wang, Dehua Huang and Roshantha A. S. Chandraratna**. "Methods of providing and using compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,313,107, November 6, 2001.

22. **Jayasree Vasudevan, Alan Johnson and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,303,785, October 16, 2001.

23. **Jayasree Vasudevan, Alan Johnson, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,291,677, September 18, 2001.

24. **Jayasree Vasudevan, Alan Johnson, Dehua Huang and Roshantha A. S. Chandraratna**. "Compounds having activity as inhibitors of cytochrome P450RAI", ***U.S. Patent***, 6,252,090, June 26, 2001.

25. **Vidyasagar Vuligonda, Kwok Yin Tsang, Jayasree Vasudevan and Roshantha A. S. Chandraratna**. "2,4-Pentadienoic acid derivatives having selective activity for retinoid X (RXR) receptors". ***U.S. Patent***, 6,147,224, November 14, 2000.

26. **Jayasree Vasudevan, Richard L. Beard, Dehua Huang and Roshantha A. S. Chandraratna**. "Amines substituted with a dihydro-benzofuranyl or with a dihydro-isobenzofuranyl group, an aryl or heteroaryl group and an alkyl group having retinoid-like biological activity". ***U.S. Patent***, 6,093,838, July 25, 2000.

27. **Jayasree Vasudevan, Vidyasagar Vuligonda, Richard L. Beard and Roshantha A. S. Chandraratna**. "Tetrahydroquinolin-2-one or 7-yl, tetrahydroquinolin-2-thione 6 or 6-yl pentadienoic acid and related derivatives having retinoid-like biological activity". ***U.S. Patent,*** 6,048,873, April 11, 2000.

Research Publications

1. **Scott M. Thacher, Jayasree Vasudevan, Kwok Yin Tsang, Sunil Nagpal and Roshantha A. S. Chandraratna.** "New dermatological agents for the treatment of psoriasis". ***J. Med. Chem.,*** 2001, 44(3), 1-17.

2. **Scott M. Thacher, Jayasree Vasudevan, and Roshantha A. S. Chandraratna.** "Therapeutic applications for ligands of retinoid receptors". ***Curr. Pharm. Design***, 2000, 6(1), 25-38.

3. **Spencer Knapp, Jayasree Vasudevan, Thomas J. Emge, Byron H. Arison, Joseph A. Potenza, and Harvey J. Schugar.** "A tethered porphyrin dimer with Π-Overlap of a single pyrrole ring". ***Angew. Chem. Int. Ed.,*** 1998, 37, 2368-2370.

4. **Jayasree Vasudevan, Robert T. Stibrany, Jean Bumby, Spencer Knapp, Joseph A. Potenza, Thomas J. Emge and Harvey J. Schugar.** "An Edge-over-edge Zn(II) Bacteriochlorin Dimer Having an Unshifted Q_y Band. The Importance of Π-Overlap". ***J. Am. Chem. Soc.***, 1996, 118, 11676-11677.

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2. "Two Modes of Self-Coordinating Edge-over-Edge Zn(II) Porphyrin Dimerization", Robert T. Stibrany, Jayasree Vasudevan, Joseph A. Potenza, Tom Emge, and Harvey J. Schugar, Poster, ***30th Middle Atlantic Regional Meeting, Villanova University***, May 1996.

3. "Two Modes of Self-Coordinating Edge-over-Edge Zn(II) Porphyrin Dimerization", Robert T. Stibrany, Jayasree Vasudevan, Joseph A. Potenza, Tom Emge, and Harvey J. Schugar, Poster, ***Eighth Annual Mini Symposium, Molecular Biophysics, Rutgers University,*** May 1996.